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PRINCIPAL INVESTIGATOR: Li-Kuo Su, Ph.D.

CONTRACTING ORGANIZATION: The University of Texas M.D. Anderson Cancer Center
Houston, Texas 77030

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Li-Kuo Su, Ph.D.

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The University of Texas M.D. Anderson Cancer Center
Houston, Texas 77030
E-Mail: lsu@mail.mdanderson.edu

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13. ABSTRACT ***(Maximum 200 Words)***

The *BRCA2* tumor suppressor gene has been suggested to play an important role in DNA repair and maintaining genome integrity. Most evidences supporting this hypothesis, however, were obtained from studying mouse embryonic stem cells or embryonic fibroblast. The importance of BRCA2 in maintaining genome integrity in human cells is not very clear. We have completed the Task 1, generation of Capan-1 derivatives that conditionally express wild type BRCA2. Capan-1 is the only human cell lines known to not express wild type BRCA2. We have obtained two Capan-1 derivatives that express exogenous wild type BRCA2 under the regulation of tetracycline. We have also obtained several Capan-1 derivatives that express exogenous mutant BRCA2, either constitutively or regulated by tetracycline. We have also carried out the first part of the Task 3, characterization of Capan-1 derivatives to genotoxic agents. We examined the sensitivity of wild type BRCA2-expressing Capan-1 derivatives to ionizing radiation and DNA damaging chemicals. Our preliminary results showed that there was no detectable difference in the sensitivity to these treatments between when these cells expressed or did not express the wild type BRCA2.

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Introduction

People carrying germline mutations of the breast cancer susceptibility gene *BRCA2* have increased risk for breast, ovarian, pancreatic and other types of cancer (Wooster et al., 1994; Wooster et al., 1995; Tavtigian et al., 1996). Mouse cells lacking a functional *Brca2* gene are deficient in repairing DNA damage (Sharan et al., 1997; Connor et al., 1997; Patel et al., 1998; Davies et al., 2001; Moynahan et al., 2001). Capan-1, a human pancreatic cancer cell line, is the only human cell line known to not express wild-type BRCA2. Capan-1 cells carry only a mutant *BRCA2* (6174delT) and expresses a truncated BRCA2 protein (Goggins et al., 1996; Teng et al., 1996; Chen et al., 1998; Su et al., 1998). The *BRCA2* 6174delT mutation is one that found frequently in Ashkenazi Jews and one that clearly predisposes its carriers to a variety of cancers (Berman et al., 1996; Neuhausen et al., 1996; Oddoux et al., 1996; Roa et al., 1996; Tonin et al., 1996; Abeliovich et al., 1997). Capan-1 cells have been shown to be more sensitive to DNA damaging agents than other human cell lines were (Chen et al., 1998; Abbott et al., 1998). However, Capan-1 cells have many additional genetic alternations compared to these other human cell lines, whether the increased sensitivity of Capan-1 cells to genotoxic agents is caused by the lack of functional BRCA2 is not clear. The goals of this study are to investigate whether alternation of the expression of wild-type BRCA2 in human cell lines would alter the ability of these cells to repair their DNA damage. We have accomplished the Task 1 of this project, to establish Capan-1 derivatives that express wild-type BRCA2. We are carrying out the first part of Task 3 of this project, to investigate whether expression of wild-type BRCA2 alters the sensitivity of Capan-1 to DNA damaging agents.

Body

1. Generation of wild-type BRCA2-expressing Capan-1 derivatives

We established tetracycline regulated wild-type BRCA2-expressing Capan-1 derivatives by transfecting a vector expressing tetracycline regulated activator, tTA-IRES-Neo (Yu et al., 1999), together with a vector expressing BRCA2 cDNA under the control of tetracycline regulated promoter. We identify wild-type BRCA2-expressing Capan-1 derivatives by using immunoblotting using the antibody N61, a BRCA2 monoclonal antibody we generated (Su et al., 1998). After screening about 140 clones obtained from two separate transfection experiments, we

isolated two clones, one from each transfection, that expressed wild-type BRCA2 tightly regulated by tetracycline (figure 1).

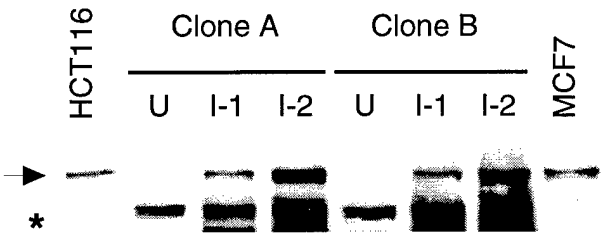
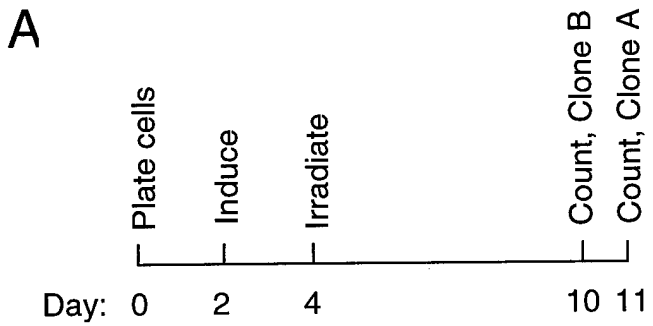


Figure 1. Expression of wild-type BRCA2 in Capan-1 derivatives.
Two clones of wild-type BRCA2-expressing Capan-1 derivative have been obtained (Clone A and Clone B). Lysates were prepared from cells grown at the uninduced condition (U), of tetracycline-containing media, or at the induced condition, tetracycline-free media, for 1 (I-1) or 2 (I-2) days. BRCA2 proteins were detected by using immunoblotting using the BRCA2 monoclonal antibody N61 (Su et al., 1998). The arrow and the asterisk indicate the wild-type BRCA2 and the Capan-1 endogenous mutant BRCA2 respectively. Lysates prepared from HCT116 and MCF7 cell lines, which expressed wild-type BRCA2, were used as positive control for wild-type BRCA2.

2. Characterization of wild-type BRCA2-expressing Capan-1 derivatives

We investigated whether expression of wild-type BRCA2 altered the sensitivity of Capan-1 cells to γ-radiation. We compared the sensitivity of these Capan-1 derivatives to γ-radiation between expressing and not expressing wild-type BRCA2. Cells were plated in ten cell culture dishes in the presence of tetracycline, therefore the wild-type BRCA2 was not expressed. The wild-type BRCA2 was induced to express in five dishes of these cells two days after plating. Another two days later, cells were irradiated with different doses of γ-radiation. Cells were grown for another six (for clone B) or seven (for clone A) days and the number of surviving cells in each dish were determined. We have done this experiment three times for clone A and two times for clone B, we are performing the third time for clone B. The results show that expression of wild-type BRCA2 did not result in detectable difference in the γ-radiation sensitivity in these Capan-1 derivatives (figure 2).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Irradiate
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Count, Clone A
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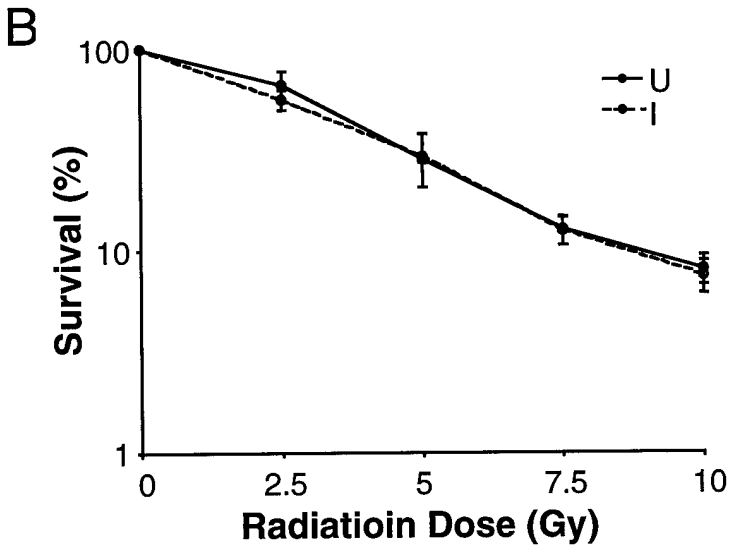
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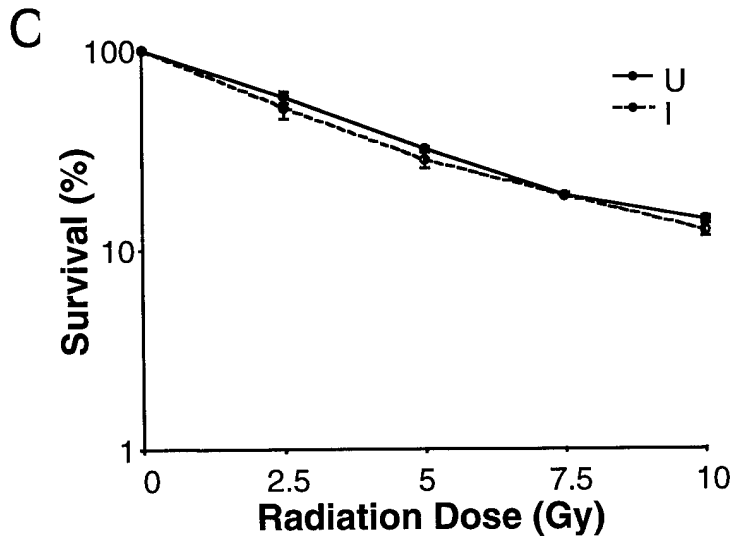
C
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Survival (%)
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5
7.5
10
Radiation Dose (Gy)

Figure 2. Sensitivity of wild-type BRCA2-expressing Capan-1 derivatives to γ-radiation. **(A)** The outline of the experiment. Five hundred thousand cells were plated in each of ten 60 mm cell culture dish on day 0. Cells were fed with fresh media on day 2, five dishes of cells were fed with tetracycline-free media to induce the expression of wild-type BRCA2. On day 4 cells were fed with fresh respective media in the morning and were irradiated with different doses of γ-radiation in the afternoon. Cells were then fed with fresh respective media every two days. Surviving cells were harvested on day 10 (clone B) or day 11 (clone A) and the number of surviving cells in each dish was determined. **(B, C)** Surviving curve of Capan-1 derivatives treated with γ-radiation. The results of three separate experiment of clone A **(B)** and two separate experiment of clone B **(C)** are shown. U and I indicate cells grown in uninduced and induced conditions, respectively.

Key Research Accomplishments

- Establishment of Capan-1 derivatives that express wild-type BRCA2 under the regulation of tetracycline

Reportable Outcomes

- Development of Capan-1 derivatives that express wild-type BRCA2 under the regulation of tetracycline
- A manuscript describing the effect of expressing wild-type BRCA2 on the growth of Capan-1 cells is being prepared
- An abstract describing the characterizing the sensitivity of these Capan-1 derivatives to genotoxic agents has been submitted to 51st Annual Meeting (year 2001) of American Society of Human Genetics

Conclusions

We have accomplished Task 1 of this project, generation of wild-type BRCA2-expressing Capan-1 derivatives. We are investigating the sensitivity of these Capan-1 derivatives to DNA damaging agents, the first part of Task 3. We are also working on the Task 2, generation of MCF-12A and MCF7 derivatives that do not express wild-type BRCA2. We will continue working on Task 2 and Task 3 in the next year.

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Appendix:
None.